

Olink™ Reveal

Laboratory instructions

For Research Use Only. Not for use in diagnostic procedures.

Before performing the protocol for the first time, please read the [Olink™ Reveal Overview User Manual](#).

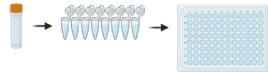
Overview

Day 1

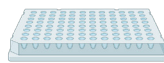
Prepare Sample Source Plate



Prepare Sample Prep Plate

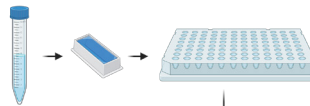


Set up Incubation



Day 2

Perform PCR



Pool PCR products



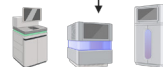
Purify library



Quality Control



Next-generation sequencing



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<https://BioRender.com/T38z823>

Important information

Unless stated otherwise, perform all steps at room temperature. We **do not** recommend using microplate vortexers.

Technical support and associated documentation

Associated documentation can be downloaded from olink.com/downloads.

For questions or guidance, please contact support@olink.com.



Day 1

Prepare bench	Storage Fill volume		
● Olink™ Explore/Reveal Negative Control	-20 °C	160 µL	Thaw at room temperature
● Olink™ Explore/Reveal Plate Control	-80 °C	160 µL	Thaw at room temperature
● Olink™ Explore/Reveal Sample Control	-80 °C	95 µL	Thaw at room temperature
● Olink™ Reveal Sample Buffer	-20 °C	1375 µL	Thaw at room temperature
Olink™ Reveal Incubation Plate	4 °C		Store at room temperature 15–20 minutes
Samples			Thaw at room temperature if frozen
2x 96-well PCR Plates			Mark one plate "Sample Source Plate"
			Mark one plate "Sample Prep Plate"
1x 8-well strip with caps			
Single-channel pipettes (P1-20, P 20-200, P100-1000)			
Multi-channel pipettes (P2-20, P10-100)			
Filter pipette tips			
Adhesive films (-80 °C compatible)			
Vortex mixer			
Mini centrifuge			
Plate centrifuge			

Prepare Sample Source Plate

1. Vortex then spin down the samples, ● Negative Control, ● Plate Control, and ● Sample Control.
2. Pipette 10 µL of the samples and controls into the Sample Source Plate according to the plate layout in ①.

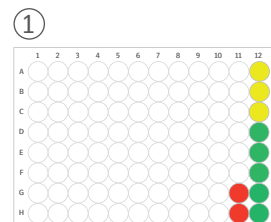
NOTE: If an alternative plate map is used, such as to randomize the locations of the Negative Controls, Plate Controls, and Sample Controls, record the locations and provide them to the NPX™ Map software later.

3. Seal the Sample Source Plate with adhesive film and centrifuge at 400–1,000 x g for 1 minute.
4. Store the Sample Source Plate at 4 °C if used the same day, otherwise at -80 °C.

Prepare Sample Prep Plate

Prepare the Sample Prep Plate no more than 1 hour before starting the incubation. Store at 4 °C until use.

5. Vortex the Sample Source Plate for 5–10 seconds then centrifuge it at 400–1,000 x g for 1 minute.
6. Vortex the ● Sample Buffer then spin it down.
7. Pipette 160 µL of ● Sample Buffer to each well of an 8-well strip tube and spin it down.
8. Reverse-pipette 12 µL of ● Sample Buffer from the 8-well strip to every well of the Sample Prep Plate. Use a multi-channel pipette without changing tips.
9. Forward-pipette 4 µL of the samples and controls from the Sample Source Plate to the corresponding well of the Sample Prep Plate. Use a multi-channel pipette and change



pipette tips between each column.

10. Seal the Sample Prep Plate with adhesive film and vortex for 5–10 seconds. Make sure that all wells are vortexed thoroughly. Centrifuge at 400–1,000 x g for 1 minute.

Set up incubation

11. Remove the Incubation Plate from the packaging.
12. Centrifuge the Incubation Plate at 400–1,000 x g for 1 minute before removing the seal.
13. Forward-pipette 4 µL from each well of the Sample Prep Plate to the corresponding well of the Incubation Plate.
 - Use a multi-channel pipette.
 - Dispense samples towards the wall of the wells as shown in ②.
 - Do not touch the dried reagents in the well with the pipette tip.
 - Change tips between each column.
14. Seal the Incubation Plate with adhesive film.
Do not vortex the Incubation Plate.
15. Centrifuge at 400–1,000 x g for 1 minute.
16. Store the Incubation Plate at room temperature in the dark for 16–24 hours.



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<https://BioRender.com/q37m709>

NOTE: When setting up for incubation, the room temperature range should be 18–27 °C. The best results are achieved when the temperature remains consistently at 25 °C. When using a thermal cycler for incubation, set the heated lid at ≥ 30 °C to prevent condensation. We recommend 105 °C. For Olink™ Reveal projects involving multiple plates, the incubation time for each individual plate should be within 1 hour.

Prepare for day 2

17. Thaw the ● PCR Additive at room temperature overnight.
18. Thaw the ● PCR Solution at 4 °C overnight.
19. Store 20 mL Milli-Q water in the fridge at 4 °C.

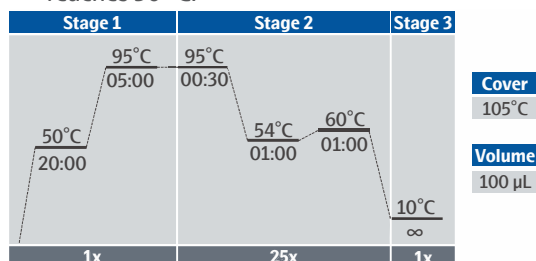


Day 2

Prepare bench	Fill volume
● Olink™ Reveal PCR Additive	1520 µL Thawed from Day 1 at room temperature. Keep at room temperature while working.
● Olink™ Reveal PCR Solution	1520 µL Thawed from Day 1 at +4°C. Keep at +4°C while working.
● Olink™ Reveal Enzyme A	320 µL Keep on freezing block at -20 °C
● Olink™ Reveal Enzyme B	50 µL Keep on freezing block at -20 °C
Olink™ Reveal Incubation Plate	Prepared from Day 1
1x 15 mL tube	Mark the tube "PCR Mix"
4x 2 mL tubes	Mark the tubes: "PCR Pool", "70% EtOH", "BP" and "Lib"
8-well strip with caps	
Reagent reservoir (25 mL)	
Multi-channel pipettes (P2-20, P10-100)	
Single-channel pipettes (P2-20, P 20-200, P100-1000)	
Filter pipette tips	
Adhesive films	
AMPure XP beads	
96% ethanol	
Milli-Q water	
Magnetic stand	
Timer	
Thermal cycler with a 96-well block	
Vortex mixer	
Mini centrifuge with rotor for 1.5 ml tubes and 8-well strips	
Plate centrifuge	

Before you start

1. Keep the ● PCR Enzyme A and ● PCR Enzyme B on a freezing block at -20 °C.
2. Ensure that the ● PCR Additive and the ● PCR Solution are thawed and have reached room temperature.
3. Set the PCR protocol as follows and start it. Pause the PCR protocol when then PCR block reaches 50 °C.



IMPORTANT: Please use this exact PCR protocol. The initial 50 °C hold facilitates the the extension step of PEA and is critical for Olink™ Reveal performance.

Day 2 (cont.)

Perform PCR

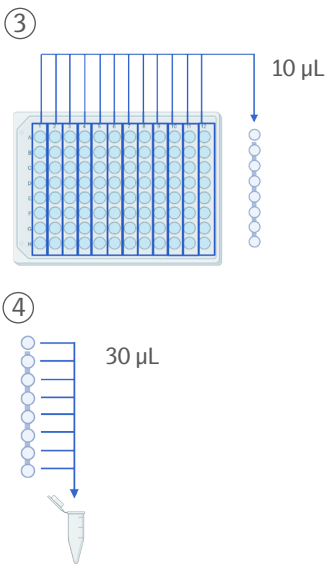
- 4. Vortex the ● PCR Solution and ● PCR Additive then spin them down.
- 5. Retrieve the incubation plate and centrifuge at 400–1,000 x g for 1 minute.
- 6. Spin down ● PCR Enzyme A and ● PCR Enzyme B briefly. **Do not** vortex them. Keep them on a freezing block at -20 °C.
- 7. Prepare the PCR Mix tube using a 15 mL tube. To ensure proper mixing, **do not** use a 50 mL tube. Add reagents according to the order listed, and invert and vortex the PCR Mix tube for 3–5 seconds where noted in the table. Invert and vortex Pipette the ● PCR Additive and the ● PCR Solution carefully from the surface due to high volume in the tubes.

Order	Reagents	Volume	
1	Milli-Q water (4 °C)	10,000 µL	Invert and vortex
2	● Reveal PCR Additive	1,310 µL	
3	● Reveal PCR Solution	1,310 µL	
4	● Reveal Enzyme A	263 µL	
5	● Reveal Enzyme B	34 µL	
Total		12,917 µL	

- 8. Pour the PCR Mix into the reagent reservoir.
 - 9. Carefully remove the adhesive film from the Reveal Incubation Plate.
 - 10. Set the timer for 10 minutes then start it. Transfer 96 µL of PCR Mix to the upper parts of each of the well walls of the Incubation Plate. Use reverse pipetting and **do not** change tips. **Do not** let the tips come in contact with contents of the wells.
 - 11. Seal the Incubation Plate with adhesive film and vortex for 3–5 seconds. Centrifuge it at 400–1,000 x g for 1 minute.
 - 12. Wait till the timer ends then place the Incubation Plate in the thermal cycler. Set the PCR protocol to resume. The protocol takes 2 hours.
 - 13. Continue to [Pool PCR products] or store the plate at 4 °C if used later on the same day.
- Safe Stopping Point: The plates can be stored at -20 °C for up to 4 weeks.

Pool PCR products

- 14. Vortex the Incubation plate for 3–5 seconds then centrifuge it at 400–1,000 x g for 1 minute.
 - 15. Pipette 10 µL from each column into one 8-well strip, one column at the time, according to ③. Use a multi-channel pipette and change tips after each column.
Please ignore precipitates that may form at this step. They do not interfere with the results and can be carried over.
 - 16. Vortex and spin down the 8-well strip.
 - 17. Pipette 30 µL from each well of the 8-well strip into the PCR Pool tube according to ④.
Change tips after each well.
 - 18. Vortex and spin down the PCR Pool tube.
 - 19. Continue to [Purify library] or store the PCR Pool tube at 4 °C if used later on the same day.
- Safe Stopping Point: The PCR Pool tube can be stored at -20 °C for up to 4 weeks.



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<https://BioRender.com/o48x036>

Day 2 (cont.)

Purify library

20. Let the refrigerated AMPure XP bottle reach room temperature.

21. Prepare fresh 70% ethanol in the 70% EtOH tube:

Milli-Q water	0.5 mL
96% ethanol	1.5 mL
Total	2 mL

22. Shake and vortex the bottle of AMPure XP vigorously to resuspend the magnetic beads.

23. Add PCR products from the PCR Pool tube and beads to the BP tube according to the table.

Mix by pipetting the contents of the BP tube up and down 10 times.

PCR products	50 µL
Magnetic beads	80 µL
Total	130 µL

24. Incubate the BP tube for 5 minutes at room temperature.

25. Place the BP tube on the magnetic stand for 2 minutes to separate beads from the supernatant.

26. Open the caps carefully and discard 125 µL of the supernatant, leaving 5 µL behind. Use a single-channel pipette. **Do not** disturb the bead pellet and **do not** remove the tube from the magnetic stand.

27. Pipette 500 µL from the 70% EtOH tube to the BP tube. Pipette onto the opposite wall from the bead pellet. **Do not** disturb the bead pellet.

28. Incubate the BP tube for 30 seconds.

29. Aspirate and discard all the ethanol using a pipette. **Do not** disturb the bead pellet.

30. Repeat steps 27-29 one more time.

31. Use a smaller pipette to remove any residual ethanol.

32. Leave the BP tube with the lid open for 2 minutes on the magnetic stand to dry the bead pellet.

33. Remove the BP tube from the magnetic stand.

34. Add 50 µL of Milli-Q water and pipette mix towards the bead pellet 10 times to resuspend it.

35. Incubate the BP tube for 2 minutes at room temperature.

36. Place the BP tube back onto the magnetic stand and leave it for 1 minute to allow the bead pellet to separate from the eluted libraries.

37. Pipette 45 µL of eluate from the BP tube while it is on the magnetic stand to the Lib tube. **Do not** disturb or aspirate the bead pellet.

38. Discard the BP tube.

Safe Stopping Point: The Lib tube can be stored at -20 °C for up to 12 weeks.

39. Store the PCR Pool tube at -20 °C in case the purification needs to be repeated.



Quality control

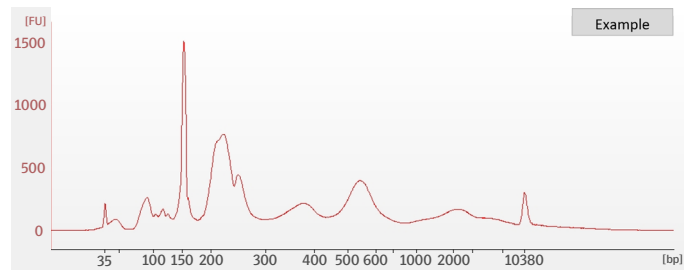
Prepare bench

- 2100 Bioanalyzer with High Sensitivity DNA Kit.
- 4200 TapeStation with High Sensitivity D1000 ScreenTape assay.
- Unless sequencing with an Illumina instrument: Qubit with 1x dsDNA HS Assay Kit.

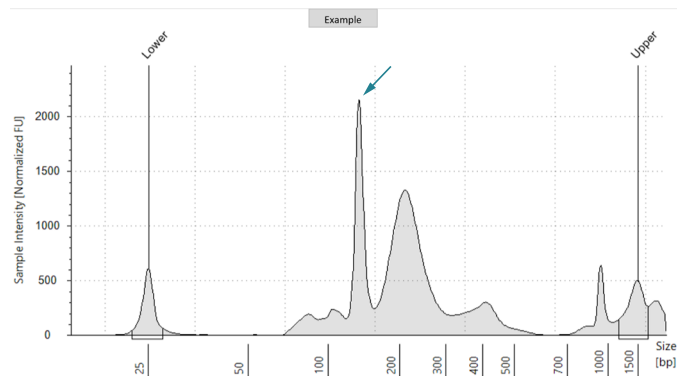
Quality control

1. Run the library on the 2100 Bioanalyzer (High Sensitivity DNA Kit) or the 4200 TapeStation (High Sensitivity D1000 Screen Tape) according to the manufacturer's instructions.
2. Check that the resulting electropherograms display a single or double peak at around 150 bp. Peaks of larger sizes represent bubble products and do not impact sequencing results.

Electropherograms Bioanalyzer



Electropherograms TapeStation



3. Quantify library concentration on the Qubit (1x dsDNA HS Assay Kit) according to the manufacturer's instructions.

NOTE: Library quantification is not required when sequencing on Illumina sequencers where fixed dilution of libraries is used. Sequencing on other sequencers uses library conversion, which requires quantification.



Illumina sequencing

The procedure for sequencing on the Illumina sequencers described below is intended for users who are already familiar with the method. Please refer to the manufacturer's instruction for full details.

Before you start

1. Transfer the required Olink custom recipe to the sequencer in advance. To obtain the custom recipe, please contact support@olink.com.
2. Install ngs2counts, based on the document NPX™ Map Preprocessing Technical Information.

Dilute libraries

3. Dilute the libraries based on the flowcell intended for use. Use the recommended dilution with either Milli-Q water or resuspension buffer (RSB, provided by Illumina) given in the table. This step replaces the library dilution in the manufacturer's instructions. When diluting libraries, the volume of purified library used should be at least 4 µL.

Sequencer	Flowcell	Dilution	Dilution buffer	Sequencer control software	Custom recipe
Illumina NextSeq 2000	P4 XLEAP-SBS Reagent Kit (50 cycles)	1:100 and then 1:8	RSB	v1.7 - v2.0	Olink_NSQ2K_P4_V2
Illumina NovaSeq X or NovaSeq X Plus	1.5B Reagent Kit (100 cycles)	1:300	Milli-Q water	v1.3.x	Olink_NovaSeqX_1.5B_1Lib_V4
	1.5B Reagent Kit (100 cycles)	1:300	Milli-Q water	v1.4	Olink_NovaSeqX_1.5B_1Lib_V5
	10B Reagent Kit (100 cycles)	1:300	Milli-Q water	v1.3.x	Olink_NovaSeqX_10B_8Lib_V4
	10B Reagent Kit (100 cycles)	1:300	Milli-Q water	v1.4	Olink_NovaSeqX_10B_8Lib_V5
Illumina NovaSeq 6000	S1 Reagent Kit v1.5 (100 cycles)	1:350	Milli-Q water	v1.8	Olink_NovaSeq6K_STND_SP_S1_V1
	S4 Reagent Kit v1.5 (35 cycles) with XP loading	1:350	Milli-Q water	v1.8	Olink_NovaSeq6K_XP_S4_V2

4. Following the corresponding manufacturer's instructions on the next steps to denature and load the libraries on to the flowcell. One individual library is added to each lane of the flowcell, except for NovaSeq X 1.5B and NovaSeq 6000 S1 flowcells, where the same library is loaded on both lanes.

IMPORTANT: The cycle numbers for all custom recipes are 24-0-0-0. Entering different cycles will lead to catastrophic loss of data. Warnings in the Illumina software that cycles have to be set to 26 or above should be ignored.

NOTE: The use of PhiX is not required.



MGI sequencing

The procedure for sequencing on MGI G400 with the FCL SE50 flow cell (MGI Cat. No. 1000014049) or MGI T1+ with the FCL SE50 flow cell (MGI Cat. No. 940-002572-00) described below is intended for users who are already familiar with these instruments. Please refer to the manufacturer's instructions for full details.

Library conversion

Olink™ Reveal has been verified on the MGI G400 (1 library per flow cell) and T1+ sequencers (2 libraries per flow cell). Libraries are converted using the MGIEasy Olink Library Circularization kit (MGI Cat. No. 940-001884-00).

1. Quantify Olink™ Reveal libraries with a Qubit 1x dsDNA HS Assay Kit (ThermoFisher).
2. Set up multiple circularization reactions in 0.2 ml PCR tubes with 60 ng Reveal library each.

Sequencer	Circularization reactions	Total amount of library
G400	2	120 ng
T1+	3	180 ng

3. Phosphorylate, circularize, digest and purify according to the manufacturer's instructions.
4. Quantify the purified, circularized ssDNA library in each reaction with a Qubit ssDNA Assay Kit (ThermoFisher) and confirm that the concentration exceeds 0.6 ng/μL.
5. Pool individual circularization reactions in one 1.5 mL tube per library.

DNB preparation and sequencing

1. Proceed with DNB preparation following the instructions in the sequencing kit manual, using the quantities specified in the table below.

Sequencer	Reaction volume	Number of DNB reactions	ssDNA amount per reaction
G400	100 μL	2 (to load 4 lanes)	8 ng
T1+	100 μL	2 (to load 2 lanes)	10 ng

NOTE: When running a single library on the T1+, perform additional DNB reactions to enable loading the same library across all four lanes.

2. Load a single Reveal library on all four lanes of a G400 FCL flow cell. The T1+ has sufficient output to sequence two libraries per FCL flow cell by adding each library to two individual lanes.
3. When setting up the run protocol on the sequencer, generate a custom sequencing protocol for Olink™ products using the following settings:
 - **Read1:** enter 66.
 - **Read2, Barcode and Dual Barcode:** Leave these fields empty.
 - **Dark reaction cycles:**
 - For **Read1:** enter 9-28, 37-58.
 - For **Read2:** Leave empty.

IMPORTANT: Entering different cycles for reads or dark reaction cycles will lead to catastrophic loss of data.

4. After sequencing, assign FASTQ files from different lanes to individual Olink™ Reveal libraries and preprocess using the **Preprocess data** function in the NPX Map software.



Element Biosciences AVITI™ sequencing

The procedure for next-generation sequencing with an AVITI™ or AVITI24™ sequencer is intended for users who are familiar with the method. Olink Reveal is compatible with the Element 2x75 Sequencing Kit Cloudbreak FS High Output (Element Biosciences Catalog # 860-00015). Libraries sequenced on Element should be processed with NPX Map according to the NPX™ Map Software User Manual.

Please refer to the Cloudbreak™ Sequencing User Guide (Element Biosciences) for full instructions.

Before you start

1. Quantify the concentration of the library on a Qubit using the dsDNA HS Assay-Kit according to the manufacturer's instructions.
2. Convert measured library concentration to pM using a Reveal library size of 150 bp.

Formula for concentration conversion:

- $\text{nM concentration} = (\text{ng/ul value from Qubit}) / (150 \text{ bp} \times 660 \text{ g/mol}) \times 10^6$
- Example: 10 ng/ul Olink library is equivalent to $10 / (150 \times 660) \times 10^6 = 101 \text{ nM}$.

Prepare libraries and configure the sequencing run

1. Prepare libraries for Cloudbreak FS sequencing according to the manufacturer's instructions. The following loading concentrations need to be used. Increased concentrations can cause higher CVs.

Instrument version	Loading concentration
AVITI	5.5 pM
AVITI24	4.0 pM

NOTE: No custom primers are needed. Steps related to sequencing with custom primers can be skipped. Olink Reveal libraries are sequenced without PhiX spike-in.

IMPORTANT: Configure the sequencing run according to the manufacturer's instructions. Enter 67, 0, 0, 0 (read 1, read 2, index 1, index 2) when prompted to define the number of cycles in each read. No custom recipe is required.

IMPORTANT: **Do not** use the "Low-Diversity High-Multiplex" option when defining run parameters. This option is incompatible with Olink Reveal libraries.

2. Sequencing data can be directly preprocessed in NPX Map according to the NPX™ Map Software User Manual.



Ultima Genomics sequencing

The procedure for sequencing Olink™ Reveal on the Ultima Genomics UG100 described below is intended for users who are already familiar with these instruments. Please refer to the manufacturer's instructions for full details.

Library conversion

Up to 10 Olink™ Reveal libraries can be sequenced on a single UG100 wafer at a time. Libraries that will be sequenced on the same wafer will need to be indexed with distinct Ultima indexing primers. Please follow the manufacturer's instructions (D1001055 - UG Indexing PCR Library Preparation Protocol for Solaris Flex) for indexing and library conversion. Briefly:

1. Quantify Olink™ Reveal libraries with a Qubit 1x dsDNA HS Assay Kit (ThermoFisher)
2. Perform indexing and amplification PCR using a compatible mastermix:
 - 20 µL Olink™ Reveal library diluted to ~0.5 ng / µL in Milli-Q water
 - Select one xGen™ Indexing Primer v2 for Ultima per Olink library. 4 unique primer plates in 96-well format are available from IDT (IDT product number: 10028412-10028415)
 - xGen™ Universal R Primer for Ultima (IDT product number 10028400)
3. Clean up amplified libraries with 1.5x sample volume of AMPure XP beads
4. Elute the library from the beads in 150 µL elution buffer
5. QC the libraries using the 2100 Bioanalyzer (High Sensitivity DNA Kit) or the 4200 TapeStation (High Sensitivity D1000 Screen Tape) and quantify with a Qubit 1x dsDNA HS Assay Kit. Calculate the molar concentration (pM) based on the size of the library peak at 150 nt (see Ultima manual for conversion equation).

Library pooling and sequencing

1. Create a Sample Sheet listing library names and barcodes using the UG_60cycles_Baseline_* sequencing recipe according to the D1001120 - UG Sample Sheet Reference Guide (Ultima)
2. Pool up to 10 converted Olink™ Reveal libraries to a total concentration of 600-800 pM in 600 µL Milli-Q water according to the D1001316 - Library QC and Pooling Reference Guide (Ultima)
3. Follow the instructions in D1001098 - User Quick Reference Guide – UG 100 Solaris Flex Workflow (Ultima) to perform pre-amplification, amplification, and sequencing workflows.
4. After sequencing, the UG100 output will be automatically processed with the Trimmer software (Ultima) to provide a hist.csv file.
5. Preprocess the trimmer output in NPX Map according to the NPX™ Map Software User Manual. Please use an unedited Ultima output folder as input for preprocessing.

Revision history

Version	Date	Sections affected	Description
1.7	2026-05-28	Day 2	Added clarification note.
		Illumina sequencing	Updated table with new information.
1.6	2026-04-01	Element Biosciences AVITI™ sequencing	New section.
		Day 2	Revised information in bench preparation table.
		Quality control	Added information.
1.5	2026-01-27	Ultima Genomics sequencing	New section.
1.4	2025-11-21	Illumina sequencing	Updated custom recipes in table.
1.3	2025-11-11	Day 1	Updated the lid temperature recommendation for thermal cycler incubation.
		Illumina sequencing	Updated version number for the Sequencer Control Software in the table.
		MGI sequencing	New section.
1.2	2025-09-25	Important information	Updated information.
		Day 1	Updated information.
		Day 2	Updated information.
		Quality control	Updated information.
		Illumina sequencing	Updated information.
1.1	2025-03-19	Illumina sequencing	Updated information.
1.0	2025-01-27	All	New

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